

DIURNAL PATTERNS OF METABOLIC VARIATIONS IN CHICK EMBRYOS¹

LELAND G. JOHNSON²

Department of Biological Sciences, Northwestern University, Evanston, Illinois

One of the most obvious characteristics of living organisms is the dynamic nature of their physiological constitution. A commonly observed aspect of this dynamic nature is cyclical variation in various physiological processes. Many of these cyclical variations disappear under laboratory constant conditions, but others are very persistent even when the organism is removed from obvious environmental timers. These cyclical phenomena exhibit periodicities of many different lengths including approximate solar daily, lunar daily, semi-monthly, monthly, annual, and longer term ones (Harker, 1958, 1964; Brown, 1959a; Webb and Brown, 1959; Cloudsley-Thompson, 1961; Bünning, 1964; Sollberger, 1965; Aschoff, 1965).

Since the total physiology of any organism is so broadly related to these cyclical phenomena, it is of potentially great interest to consider the ontogeny of physiological periodicity. A basic question is whether an organism's physiology possesses cyclical qualities only in a relatively mature state or whether systematic variability can be demonstrated throughout the life-history of the organism. The former seems to be the case for some overt patterns of cyclical variability such as the human infant pulse rate and sleep-wakefulness cycles (Hellbrügge, 1960). However, since these functions become phased to obvious daily environmental fluctuations and are not observed in early developmental stages, it is of interest to seek periodicities which might exist under constant conditions and in earlier developmental stages.

Hiebel and Kayser (1949) were unable to detect a clear diurnal rhythm of movement of the chick embryo within the egg. They were measuring movements of the whole egg and were seeking a clear-cut nocturnal reduction in activity. Following hatching they could detect no diurnal rhythm in heat production unless they subjected the young chicks to alternating light and dark schedules.

Aschoff and Meyer-Lohmann (1954), however, recorded motor activity of newly hatched chicks and by summing all activity in four-hour periods could show, under constant conditions, a clear rhythmic variation in activity present from the time of hatching. This rhythm had an average period of 25.4 hours. Hoffman (1957, 1959) has shown an activity rhythm of approximately 24 hours for newly hatched lizards. Petren and Sollberger (1953) found that rhythmic fluctuations in liver glycogen content were already established in very young chickens (measurements made on days 5-6, 12-13, and 36-37 post-hatching). Further studies showed that a periodic fluctuation in glycogen content was already present on the

¹ This work is a portion of a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree from the Department of Biological Sciences, Northwestern University, Evanston, Illinois.

² Present address: Department of Biology, Augustana College, Sioux Falls, South Dakota 57102.

first day after hatching and that a rhythm of somewhat different character could be demonstrated even if the newly hatched chicks were starved. In other experiments, freshly laid eggs were stored and incubated in total darkness until the twentieth day of incubation when liver glycogen determinations were made. A clear daily rhythm with a major peak and a minor peak of glycogen content was demonstrated. Therefore, the diurnal liver glycogen rhythm is well established before the time of hatching even under constant conditions.

These data relate, however, to rather advanced stages of development. One study carried out in earlier stages of development was the measurement of metabolic variations by Barnwell (1960). He was able to detect a significant mean solar daily fluctuation in rate of oxygen consumption. By measuring oxygen consumption under constant conditions, and treating data to correct for the increasing overall rate of oxygen consumption, such fluctuations were established to be present on the fifth through the eighth days of incubation (eggs were incubated at 4 PM and the day upon which incubation was begun was designated as day one).

Barnwell's work has been criticized (Heusner, 1963, 1965; Heusner and Zahnd, 1963) largely on the basis of suggested faults in his techniques. These criticisms appear to have originated largely from misinterpretations of experimental procedures and statistical techniques used by Barnwell, including underestimation of the sensitivity of his respirometers, and have been dealt with more extensively elsewhere (Johnson, 1965).

The presence of a mean daily cycle of variation in rate of oxygen consumption during these stages of development raises some new questions. It must be determined whether this periodicity has a definite pattern of ontogeny. The properties of metabolic periodicity in a developing organism should be compared with some well-known properties of other rhythmic systems. In order to make meaningful comparison, a consistent statistical treatment must be developed. Once such a treatment is available, questions about ontogeny of periodicity, seasonal cycle form differences, and temperature relationships should be answerable.

METHODS AND MATERIALS

Fertile White Leghorn eggs were obtained from a local commercial supplier and stored in a cold chamber at approximately 10° C. until used (some were incubated directly). Before incubation the eggs were allowed to warm to room temperature. Incubation was initiated at 9 PM (CST) for all experiments described here unless stated otherwise. The incubator was a forced draft model provided with water pans and set at 38° C.

Respiration was measured using Brown automatic recording respirometers (Brown, 1954, 1957). The six respirometer units were constructed at the beginning of the study.

In operation the sealed respirometer chamber was evacuated to produce an internal pressure of 28.7 inches Hg which then remained constant throughout each recording period. Temperature was controlled at 38° C. by a 55-gallon circulating water bath which surrounded the respirometer chamber, or barostat. A mercury switch and relay controlled a 450-watt immersion heater suspended in the bath. Water temperature cycled within a range of $\pm 0.05^\circ$ C. The experiments were conducted in a photographic darkroom in which the only light source was a single

ceiling fixture controlled through a voltage-regulator. The intensity of illumination reaching the interior of an uncovered barostat was less than one foot candle. With the glass cover in place, the respirometer in the interior was provided with a substantially lower level of constant dim light.

The respirometer flask itself was modified as described by Barnwell (1960), with minor changes. The support frame which held the egg in place was a cylinder produced by cutting a section out of a glass vial. The cylinder was perforated at several points to permit free passage of air under and around the egg. The egg was set on the support frame with the air chamber uppermost. A KOH solution in the bottom of the flask was the CO₂ absorbent and also served to meet humidity requirements. A single egg was suspended in the diver below the recorder and oxygen consumption values were calculated in ml. consumed per egg per hour.

Control experiments for the apparatus were conducted at the beginning of the experimental period and at various times thereafter. Extended recordings of the

TABLE I
Comparison of rates of O₂-consumption as ml. of O₂/egg/hour at several stages of incubation as determined in various studies

Hrs. incub.	This study (spring)	I*	II	III	IV	V	VI	VII	VIII
72	0.283±0.055†	0.266±0.066	0.253±0.040					0.216	
96	0.445±0.105	0.427±0.110	0.381±0.100	0.34±0.11				0.333	
120	0.715±0.101	0.680±0.131	0.524±0.025	0.44±0.05			0.612	0.628	
144	1.071±0.199	0.826±0.178	0.831±0.134	0.89±0.07	0.91±0.06		1.542	0.922	0.883
168	1.752±0.162	1.423±0.191	1.421±0.223		1.19±0.14	1.19	2.075	1.775	1.168
192	2.221±0.145	1.922±0.173	1.671±0.173	1.23±0.26	1.51±0.03	1.52	2.262	2.428	1.835

* I Barnwell, 1960; II Romanoff, 1941a; III McLimans, Siem and Scholljegerdes, 1950—Series A; IV McLimans, Siem and Scholljegerdes, 1950—Series B; V McLimans, Siem, Mark and Pinska, 1950; VI Greiff and Pinkerton, 1948; VII Hasselbalch, 1900; VIII calculated from Murray, 1925, 1926.

† Standard deviation.

behavior of weighted blank divers were made. Even when runs were continued for periods longer than any of the experimental runs, no deviations of the recording pens were detected. Since the recorders in the control runs were set at sensitivities equal to the greatest ones used in the experiments, it could safely be assumed that no oxygen loss which was detectable by the methods used occurred during these experiments.

This study was concerned more with variations in rates of oxygen consumption than with absolute rates. However, comparative data concerning absolute measurements made with this apparatus have been compiled in Table I.

One problem in determining daily variations in oxygen consumption in chick embryos is the increasing overall rate of consumption. In order to examine hourly fluctuations on a comparative basis, a correction for this trend must be made. The method chosen for this study was the construction of a line of least squares fit by regression analysis (Simpson, Roe and Lewontin, 1960). Regression analysis was performed on every complete organism-calendar-day of data collected during the

course of the study. The resultant calculated values provided a basis for determining hourly deviation from trend. Each recorded hourly value was divided by the appropriate regression value to permit its description as a percentage of trend value. These percentage values made plotting of daily values on a non-sloping line possible and allowed more valid comparisons because all data were reduced to a common relationship. Therefore, despite differences in total oxygen consumption among individual organisms on a given day of incubation and between different days of incubation, meaningful examinations of similarities and differences in form of individual patterns and mean patterns of groups could be made. Unless otherwise stated, all results and discussion presented will relate to analysis of these regressional relationships.

Several devices were employed in examining the daily patterns. Weighted (1, 2, 1) moving means (Croxtton and Cowden, 1955) were calculated from the hourly means in some instances. Means for consecutive three-hour periods were calculated in other cases. Ratios of the number of hourly recorded values falling above the trend line to the number falling below for given periods were also calculated.

RESULTS

The use of regression analysis and the calculation of per cent of trend values from data such as those collected in this study reduce all data to a common form and also make possible gross comparisons of relationships to the trend line. Since this analysis corrects for the continually increasing overall rate of oxygen consumption during ontogeny, it is possible to detect metabolic variations not accountable by simple ontogenetic increase. As the metabolism of the adult chicken has a strong diurnal variation, the per cent of trend values were first examined on the basis of an arbitrary separation of nighttime from daytime values (all 38° C., 9 PM incubation time data were used). In the 1 AM to 6 AM data 724 hourly values were found

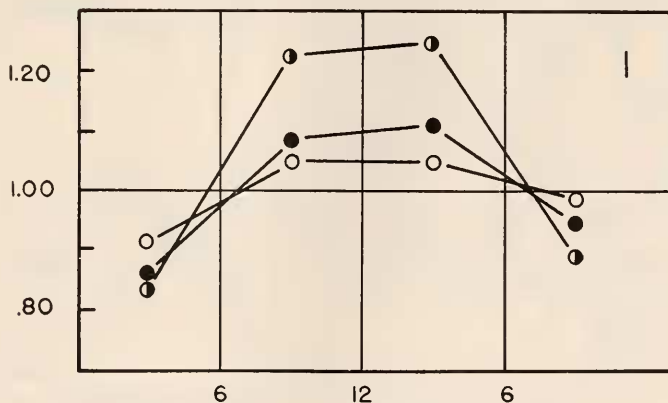


FIGURE 1. Ratios of the number of hourly values greater than 100% of trend values to the number of values less than 100% of trend calculated for six-hour periods. Closed circles represent all of the data gathered at 38° C. from embryos which were incubated initially at 9 PM (see text). Open circles represent the same data minus day-seven and day-eight data. Half-closed circles represent data gathered at 33° C.

to be above the trend line and 836 values were below it. In the 7 PM to midnight data 763 values were above and 797 values were below the trend line. The twelve daytime hours (7 AM-6 PM) showed a considerably different picture with 1624 values above 100% of trend and 1496 below it. Statistical comparison of the day and night values by use of Chi-square analysis indicates a significant difference ($\chi^2 = 12.04$, $P < 0.001$). Therefore, irrespective of any other complexities of form in the daily cycles, there appears to be a mean diurnal variation even at these early stages of development.

More refined examinations of these relationships can be accomplished in another way. A ratio of the number of hourly per cent of trend values falling above 100% of trend to those falling below that level provides a convenient numerical value for purposes of comparison. Figure 1 (closed circles) shows the ratios calculated using all hourly values for six-hour periods of the day.

The foregoing information is of value only in detecting gross relationships and it becomes necessary to use the actual per cent of trend values for more precise examination of characteristics of this metabolic variability. Ratios such as those seen in Figure 1 assign equal weight to all hourly values and do not take large percentage deviations into account. Separation of the data into three-hour periods using mean per cent of trend values yields some interesting differences among the days of incubation studied (Fig. 2). The patterns for days four, five and six show considerable similarities through the pre-dawn hours, but then a difference appears. Days four (2a) and five (2b) go on to afternoon highs and evening lows but a similar progression through the day is lacking in the day-six data (2c) except for

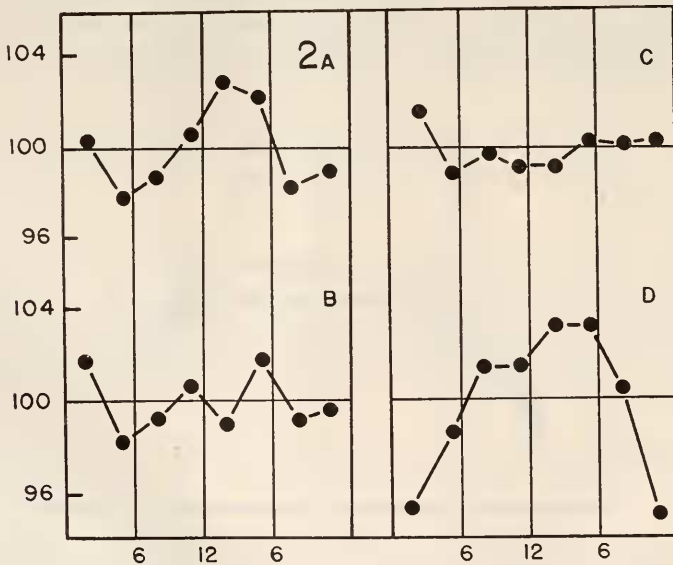


FIGURE 2. Means of hourly per cent of trend values calculated for three-hour periods of the day. The number of organism-calendar-days of data involved in each instance is indicated in parentheses. A. 38° day-four data (77). B. 38° day-five data (90). C. 38° day-six data (79). D. Combined 38° day-seven and day-eight data (12 day sevens and 7 day eights).

the merest suggestion. In the day-seven and day-eight data (2d) a new pattern is emergent. This seems to be a more emphatic expression of the gross pattern of day-night differences seen in the embryo throughout the period investigated. Since the data for days seven and eight are included in the ratios cited in Figure 1

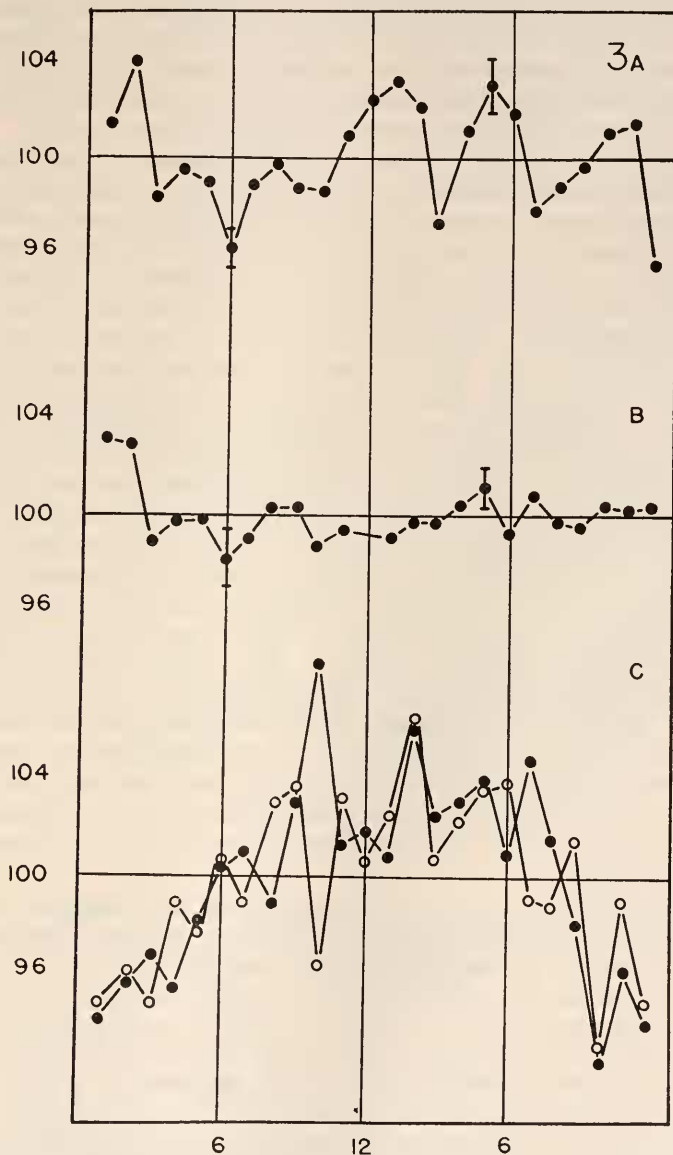


FIGURE 3. Hourly means of per cent of trend values. A. Day-four and day-five data from three seasons. B. Day-six data from three seasons. C. Day-seven (open circles) and day-eight (closed circles) data. Bars indicate standard errors of means.

(closed circles), the validity of this statement might be open to question. However, the same comparison can be made between the day-seven and day-eight pattern and that seen in the ratios which exclude day-seven and day-eight data (Fig. 1, open circles).

Figure 3 permits examination of these variations on an hour-by-hour basis. Because of the similarities seen in the patterns of variation in the day-four and day-five data, the values from both days are grouped together in Figure 3a. The apparently unique pattern of variation around the trend line seen in the day-six data is seen again in Figure 3b. The data from days seven and eight are plotted separately in Figure 3c to disclose their similarity to one another.

Another matter of concern in determining the nature of metabolic variability during development was the possibility that the daily cycle might have a different form at different times of the year. For all seasons the greatest number of uninterrupted respirometer-days was obtained for the fifth day of incubation. This day of incubation can serve as an index of seasonal fluctuation as the data should be fully comparable. Means of the per cent of trend values for three-hour periods of day five are presented in Figure 4 (a, b and c). Similarities are seen in the early morning and afternoon relationships, but a striking inversion in the morning values is seen with the spring means varying in a manner different from the fall and winter means. A comparable seasonal difference was evident for the fourth day of incubation. In order to show the daily cycle in day-five embryos in more detail, three-hour weighted (1, 2, 1) moving means of the hourly per cent of trend values are presented in Figure 5 (a, b and c). Differences among the mean daily cycles become obvious upon examination of this figure. The early morning hours (1 AM–4 AM values) in all three cases show a steady downward progression which begins well above the trend line. However, the similarity largely ceases here. In the spring, one of the highest values of the day occurs at 8 AM while a similar conspicuous increase is lacking in the fall and winter data. The pattern through the mid-part of the day in the spring is essentially inverted relative to that of the fall and winter data. In the fall and winter, a maximum occurs over the noon hour. A common characteristic of the daily cycles is a mid-afternoon low value which is seen in the data for all three seasons. This low value is followed in all cases by a sharp rise to a late afternoon high value occurring at 4, 5, or 6 PM depending upon the season of the year. The rate then drops off into an evening pattern which is similar in all seasons.

Experiments designed to reveal information about the temperature relationships of this metabolic variability were conducted in the fall. Control respirometers were run at 38° C. while other respirometers were maintained at 33° C. Eggs were transferred to both the control and the 33° C. respirometers on the third day of incubation. Data recorded on the fifth day will be emphasized here. At the beginning of day five the embryos in the 33° C. respirometers have already been subjected to 32 or more hours at the depressed temperature. Initial comparisons with control data from day five can be made by comparing Figures 6a and 5b. The daytime patterns of O_2 -consumption variation are quite similar (note 6 AM–8 PM values), but bear a different relationship to the 100% of trend line. This is due to the fact that at 33° C. the early morning and late evening values are markedly depressed and this depression is detected statistically in the form of changed rela-

tionships to the trend line. This early morning and late evening depression will be discussed more extensively later, but it should be borne in mind when focusing attention on the three-hour mean values (Fig. 4d). If the plots are compared point by point, it can be seen that the pattern of variation follows that of the control data (Fig. 4b) quite well, but the first predawn value and the two evening values are lowered in relation to the trend line. It should be noted especially that the direction of change from one value to the next is parallel in all cases except the one involving the first predawn value. Another aspect of the data taken at 33° C. to be noted is the similarity of the patterns of variation on day five and day six (Figs. 6a and b, 4e and d). Finally, the changed relationships to the trend line are obvious in the ratios of hourly values above 100% of trend to values below 100% which are presented in Figure 1 (half-closed circles). This is further indication of the relatively greater depression of the nighttime oxygen consumption by the lower temperature.

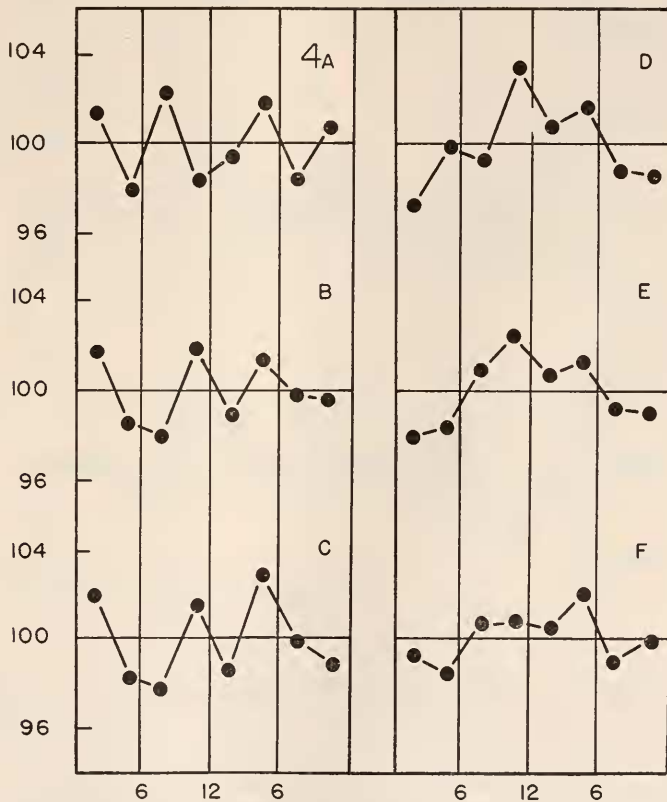


FIGURE 4. Means of hourly per cent of trend values calculated for three-hour periods of the day. The number of organism-calendar-days of data involved in each instance is indicated in parentheses. A. Spring 38° day five (30). B. Fall 38° day five (38). C. Winter 38° day five (22). D. Fall 33° day five (33). E. Fall 33° day six (35). F. Winter "9 AM" day five (16).

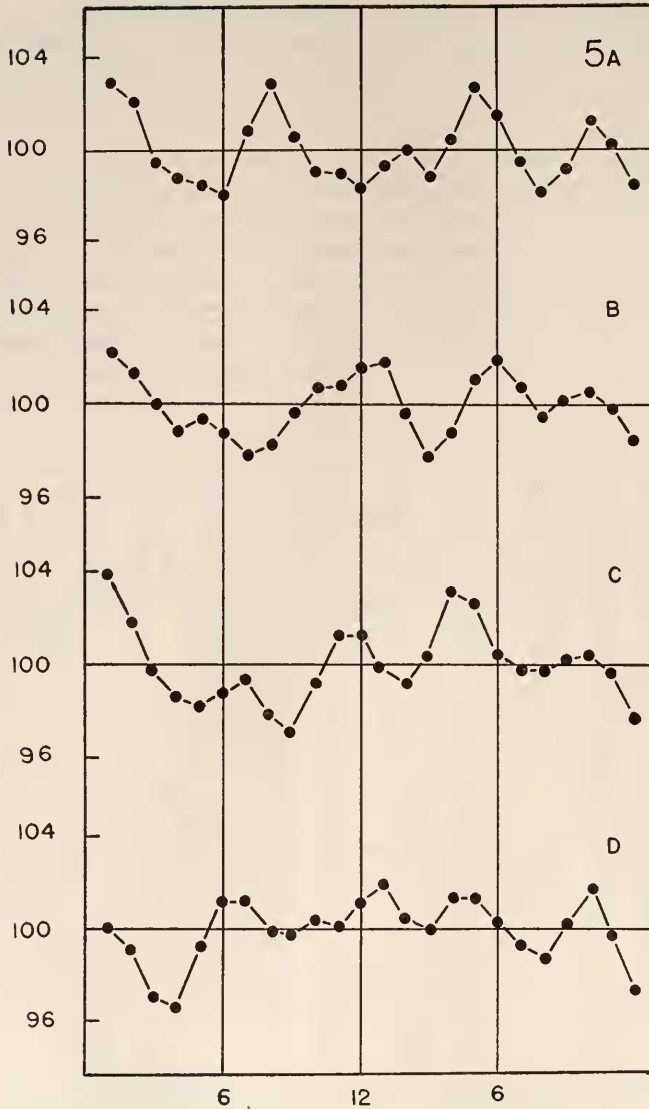


FIGURE 5. Three-hour weighted moving means (1, 2, 1) of hourly per cent of trend values. A. Spring 38° day five. B. Fall 38° day five. C. Winter 38° day five. D. Winter "9 AM" day five.

Data on the nature of metabolic variability when incubation was initiated at a different time of the day were obtained in winter. They are not as extensive as might be desired since only two times of incubation were considered. Embryos whose incubation was initiated at 9 AM were compared with embryos incubated, as in all previous experiments, at 9 PM. Only 16 complete organism-calendar-days of data were obtained for the "9 AM" group, but some suggestive conclusions are

possible. Again using day-five data for comparison it can be seen in the three-hour mean plots (Fig. 4f and c) that the pattern of variability is similar. For purposes of general comparison, attention is also called to the similarity between the "9 AM" day-five pattern and the overall mean day-five pattern (Fig. 2b). The weighted moving mean plots (Fig. 5d and c) show parallels in the early morning hours where the trend is downward. This is followed by a peak over 6 and 7 AM, a depression in mid-morning, and a peak over the noon hour. The remainder of the day's pattern can be followed in the same way. No explanation other than small

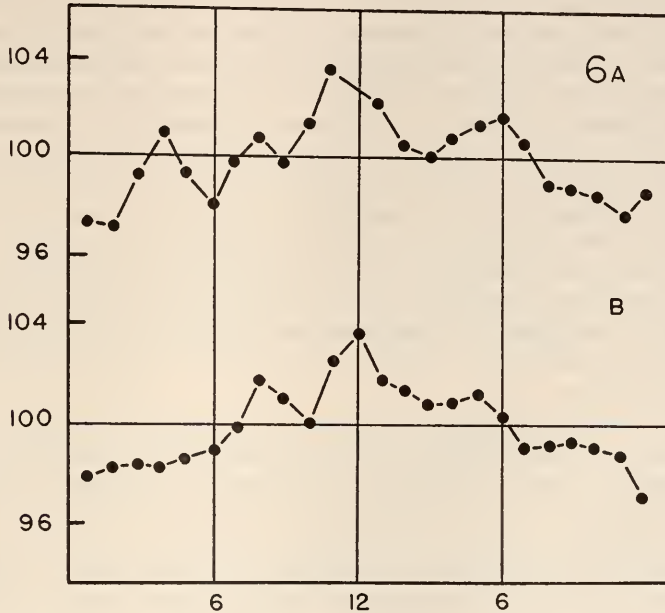


FIGURE 6. Three-hour weighted moving means (1, 2, 1) of hourly per cent of trend values.
A. 33° day five. B. 33° day six.

sample size is readily apparent for the altered relationships to the trend line, but the emphasis is placed on similarity of pattern.

DISCUSSION AND CONCLUSIONS

The data gathered in this study confirm the conclusion of Barnwell that systematic metabolic variations exist in the chick embryo as early as the first week of incubation. The present investigation of this phenomenon involved study of the forms of the daily patterns of variation from the fourth through the eighth days of incubation. The pattern seen during days four and five is one in which several peaks occur, but on days seven and eight there seems to be a somewhat simpler pattern while the pattern for day six is peculiar to that day. This could represent an evolution toward the form of the adult chicken metabolic rhythm which has been reported to be strongly diurnal (Bacq, 1929; Barott *et al.*, 1938). The nature of

the changes in the pattern of oxygen consumption seen through this period of development can only be described and any explanations of these changes must be deferred. However, it may be postulated that during this investigated period of development some overt, energy-requiring process becomes coupled into a genetically determined relationship with the underlying rhythmicity reflected in the early basal metabolic variability. If this coupling were a gradual process rather than an instantaneous one, it would provide one explanation of the failure of the day-six data to fit the pattern of either the days which precede or follow that day. Although it is possible that several or many processes might be involved in such a coupling, it has been reported that some important neuromuscular integrations are being established at about these stages of development (Hamburger, 1963; Hamburger and Balaban, 1963). Energy-requiring processes having a periodicity adopted from underlying rhythmicity might be related to this neuromuscular integration. Limb motility could be such a process. This basic rhythmicity is presumed to have been present from earliest stages of development and is available to the organism as a means of regulating various physiological processes. The organism appears to function to a certain extent as an amplifier and to produce large scale differences with periodisms adopted from this subtler underlying rhythmic system. The adult may function even more strongly as an amplifier system and this rhythmicity could be utilized to time numerous physiological events. By the time that the energy requirements of all of these processes have been met it would no longer be possible to use metabolic variability as an index of the true character of the basic rhythmic nature of the organism.

Results obtained during different seasons indicate that there is an annual component in the expression of the fundamental metabolic variability of the chick embryo. Study of a comparable stage of development (day five) at different times of the year demonstrates a seasonal variation in the form of the daily pattern of oxygen-consumption. It was noted that the daily patterns were very different from one another in spring and fall. Although the winter data were more similar to the fall data, in some ways they appeared suggestively to be intermediate between the spring and fall data. The pattern of annual variation seen in the three-hour means shows similarities to the pattern of annual variation reported for some other organisms. One of the most noticeable elements of seasonal differences is the marked change of pattern over midday. Such a seasonal noon difference was reported for the daily metabolic pattern in the potato. In that organism the noon values are relatively low points in the daily cycle during the spring and high points in the fall of the year (Brown, 1958, 1960). The inversion of the late morning mean (this mean includes the noon value) which occurs between the spring and fall data produces a similar picture in the chick embryo. The late afternoon maximum is very persistent in all seasons examined, just as reported for the potato. In organisms such as the potato (Brown, 1958) and bean (Lutsch, 1962) another aspect of seasonal differences is the variation in mean overall rate of oxygen consumption. Although very suggestive data indicating higher metabolic rates in the spring were obtained during this study, this aspect of metabolic variability in the chick embryo will require further work and such experiments have been planned.

Study of temperature relationships of the metabolic fluctuations produced some interesting results. Several lines of evidence indicate that the period length of the

basic metabolic rhythm is not altered by lowering the temperature of the organism. Although the temperature of the embryos had been lowered for 32 hours or more at the beginning of day five, fluctuations parallel those seen in the 38° C. organisms. This is particularly apparent in the pattern of variation seen in the daytime hours. Another interesting parallel is seen upon comparing the patterns of variability seen in the 33° C. day-five and day-six embryos (Fig. 6). These embryos on succeeding days show a remarkably similar pattern of variation. A question might be raised about the relationship of these data to those presented on ontogeny of the pattern of metabolic variability. It must be remembered in this connection that by the beginning of what is being called day six here the 33° C. embryos have been subjected to the lowered temperature for 56 hours or more and it is very difficult to say to what extent the previously discussed ontogenetic processes have been retarded by the lowered temperature. The conclusion drawn from these data is that the period of the metabolic rhythmicity seems to be temperature-independent or, at least, that the Q_{10} value for the period length is so close to 1.0 that any difference from that value is too small to be detected by the methods used in this study.

A second result of the lowered temperature is the suppression of the rate of oxygen consumption in the early and late portions of the 24-hour period (nighttime values) relative to the other values (daytime values). This suppression is detected through linear regression analysis as an altered relationship to the trend line. It is an interesting conjecture that even at these stages of development, there might be a regular periodic variation in the sensitivity of the organism's overall metabolism to temperature differences.

The similarity of the pattern of variation in organisms whose incubation was initiated at different times of the day demonstrates that there is no triggering associated with the beginning of incubation, but rather that the characteristic daily pattern will find expression even if the time of incubation is different. The patterns of variation shown by the "9 AM" chicks were not 12 hours out of phase with the "9 PM" chicks, but rather were in phase with them and with the time of day even under laboratory constant conditions. The differences observed between the small sample of "9 AM" chicks (16 organism-calendar-days) and the controls are probably due to individual variation or to the fact that ontogenetic factors previously discussed may obscure the results since the "9 AM" chicks are 12 hours younger in incubation age than the "9 PM" chicks. In this connection, attention is called to the overall pattern of variation in day-five embryos (Fig. 2b) and also to the day-four pattern (Fig. 2a).

The properties of this metabolic variability are of interest in themselves because of their implications concerning the ontogeny of periodicity, but they also have some bearing on more general aspects of the biological clock problem. A long-standing controversy in this field has been the support by various workers in the field of two different hypotheses concerning the nature of the timing mechanism of biological rhythms. The hypothesis of exogenous timing related to geophysical environmental factors has been supported by Brown and others (Brown, 1959b, 1960, 1962) in recent years. An alternative hypothesis is that expressed by Pittendrigh and others (*e.g.*, Pittendrigh and Bruce, 1957, 1959; Aschoff, 1963) which views the timer mechanism of biological clocks as an autonomous, endogenous oscillator

system which is physico-chemical in nature. The merits of, and evidences for, these viewpoints have been discussed extensively in a recent work by Brown (1965).

The possession of seasonal differences in the daily patterns of variability has some bearing on this question. Chick embryos whose development is initiated at one time of the year and others initiated at other times of the year show decidedly different patterns of daily variation in oxygen consumption and these seasonal patterns parallel those seen in other organisms. In the case of an autonomous internal timer, the genetic mechanisms which must be hypothesized to account for such seasonal differences would have to be fantastically complex. It seems simpler and more direct to hypothesize a basic responding system which when it is examined, even in embryonic stages, shows seasonal patterns of response reflecting seasonal differences in variations in geophysical factors.

The significance of the relative temperature-independence of the period-length of biological rhythms has been discussed extensively in the past. Proponents of the autonomous internal oscillator hypothesis assume that such an inherent oscillator system would be capable of temperature compensation. Problems are posed for this viewpoint because this type of temperature-independence is not ordinarily seen in biological chemical reactions where Q_{10} values of 2.0–3.0 and even higher are common. An alternative explanation which is consistent with the observed facts could be that a basic responding system exists and that external time cues continue to be available to the organism with regularity, no matter what the temperature of its immediate environment.

The apparent lack of a triggering point demonstrated by the experiments where incubation was initiated at different times of the day provides another piece of evidence related to the nature of the biological clocks. One group of embryos was essentially 12 hours out of phase with the other group in their relationship to the actual time of day at the beginning of incubation. The strikingly similar patterns of metabolic variations seen in the two groups is again suggestive of an open receptor system which responds to an input related to the exact time of day even under laboratory constant conditions.

I am grateful to Professor Frank A. Brown, Jr. for his advice and constructive criticism during the course of this study. Work in Professor Brown's laboratory was supported by grants from the National Science Foundation (GB-469) and the National Institutes of Health (GM 07405) and by a contract with the Office of Naval Research (1228-30). I was supported by a National Science Foundation Predoctoral Fellowship.

SUMMARY

1. Continuous recordings of oxygen consumption were made in order to determine ontogenetic, daily, seasonal, temperature, and other relationships of metabolic variability in chick embryos.

2. Statistical treatment involving linear regression analysis facilitated resolution of daily cycle forms and allowed various comparisons.

3. The pattern of metabolic variation during days four and five has several peaks, but by days seven and eight a more markedly diurnal pattern appears. Day six appears to be an intermediate or transitional stage.

4. Seasonal differences in the form of the daily cycle resemble those reported for other organisms.
5. Lowering the temperature 5° C. does not affect the period length of the metabolic variations. A suppression of nighttime metabolism relative to daytime metabolism suggests the expression of a diurnal variation in temperature sensitivity.
6. Initiation of incubation at different times of day does not result in different basic cycle forms.
7. Results obtained with chick embryos are suggestive of a receptor system which is responsive to external time cues.

LITERATURE CITED

- ASCHOFF, J., 1963. Comparative physiology: diurnal rhythms. *Ann. Rev. Physiol.*, **25**: 581-600.
- ASCHOFF, J., ed., 1965. *Circadian Clocks*. Amsterdam, North-Holland Publishing Company.
- ASCHOFF, J., AND J. MEYER-LOHMANN, 1954. Angeborene 24-Stunden-Periodik beim Kücken. *Pflügers Arch.*, **260**: 170-176.
- BACQ, Z. M., 1929. Sur l'existence d'un rythme nycthemeral de metabolisme chez le coq. *Ann. Physiol. Physicochimie Biol.*, **5**: 497-511.
- BARNWELL, F. H., 1960. A solar daily variation in oxygen consumption of the embryonated egg. *Proc. Soc. Exp. Biol. Med.*, **105**: 312-315.
- BAROTT, H. G., J. C. FRITZ, E. M. PRINGLE AND H. W. TITUS, 1938. Heat production and gaseous metabolism of young male chickens. *J. Nutrition*, **15**: 145-167.
- BROWN, F. A., JR., 1954. Simple, automatic, continuous-recording respirometer. *Rev. Sci. Instr.*, **25**: 415-417.
- BROWN, F. A., JR., 1957. Response of a living organism, under "constant conditions" including pressure, to a barometric-pressure-correlated, cyclic, external variable. *Biol. Bull.*, **112**: 288-304.
- BROWN, F. A., JR., 1958. An exogenous reference-clock for persistent temperature-independent, labile biological rhythms. *Biol. Bull.*, **115**: 81-100.
- BROWN, F. A., JR., 1959a. The rhythmic nature of animals and plants. *Amer. Scientist*, **47**: 147-168.
- BROWN, F. A., JR., 1959b. Living clocks. *Science*, **130**: 1535-1554.
- BROWN, F. A., JR., 1960. Response to pervasive geophysical factors and the biological clock problem. *Cold Spring Harbor Symp. Quant. Biol.*, **25**: 57-71.
- BROWN, F. A., JR., 1962. Extrinsic rhythmicity: a reference frame for biological rhythms under so-called constant conditions. *Ann. N. Y. Acad. Sci.*, **98**: 775-787.
- BROWN, F. A., JR., 1965. A unified theory for biological rhythms. In: *Circadian Clocks*. J. Aschoff, ed., pp. 231-261. Amsterdam, North-Holland Publishing Company.
- BÜNNING, E., 1964. *The Physiological Clock*. Berlin, Springer-Verlag.
- CLOUDSLEY-THOMPSON, J. L., 1961. *Rhythmic activity in animal physiology and behavior*. New York, Academic Press.
- CROXTON, F. E., AND D. J. COWDEN, 1955. *Applied General Statistics*. 2nd ed., Englewood Cliffs, New Jersey, Prentice-Hall, Inc.
- GRIEFF, D., AND H. PINKERTON, 1948. Effect of enzyme inhibitors and activators on the multiplication of typhus rickettsiae. III. Correlation of effects of PABA and KCN with O₂-consumption in embryonate eggs. *J. Exp. Med.*, **87**: 175-197.
- HAMBURGER, V., 1963. Some aspects of the embryology of behavior. *Quart. Rev. Biol.*, **38**: 342-365.
- HAMBURGER, V., AND M. BALABAN, 1963. Observations and experiments on spontaneous rhythmic behavior in the chick embryo. *Dev. Biol.*, **7**: 533-545.
- HARKER, J. E., 1958. Diurnal rhythms in the animal kingdom. *Biol. Rev.*, **33**: 1-52.
- HARKER, J. E., 1964. *The Physiology of Diurnal Rhythms*. Cambridge, Cambridge University Press.
- HASSELBALCH, K. A., 1900. Ueber den respiratorischen Stoffwechsel des Hühnerembryos. *Skand. Arch. Physiol.*, **10**: 353-402.

- HELLBRÜGGE, T., 1960. The development of circadian rhythms in infants. *Cold Spring Harbor Symp. Quant. Biol.*, **25**: 311-323.
- HEUSNER, A., 1963. Analyse de la variation nycthemeral du metabolisme energetique chez le rat blanc. Doctoral Dissertation, Strasbourg.
- HEUSNER, A., 1965. Sources of error in the study of diurnal rhythm in energy metabolism. In: *Circadian Clocks*. J. Aschoff, ed., pp. 3-12. Amsterdam, North-Holland Publishing Company.
- HEUSNER, A., AND J. P. ZAHND, 1963. Etude de la consommation d'oxygene de l'embryon de poulet au cours du nycthemere. *C. R. Soc. Biol., Paris*, **157**: 1498-1501.
- HIEBEL, G., AND C. KAYSER, 1949. Le rythme nycthemeral de l'activité et de la calorification chez l'embryon de poulet et le jeune poulet (Light Sussex). *C. R. Soc. Biol., Paris*, **143**: 864-866.
- HOFFMANN, K., 1957. Angeborene Tagesperiodik bei Eidechsen. *Naturwiss.*, **44**: 359-360.
- HOFFMANN, K., 1959. Die Aktivitätsperiodik von im 18- und 36-Stunden-Tag erbrüteten Eidechsen. *Zeitschr. vergl. Physiol.*, **42**: 422-432.
- JOHNSON, L. G., 1965. Studies on metabolic variations in embryonated chicken eggs at several developmental stages. Doctoral Dissertation, Northwestern Univ., Evanston, Illinois.
- LUTSCH, E. F., 1962. Rhythmic variations of oxidative metabolism in bean seedlings. Doctoral Dissertation, Northwestern Univ. Evanston, Illinois.
- McLIMANS, W. F., R. A. SIEM AND V. R. SCHOLLJEGERDES, 1950. A physiological study of virus parasitism. I. A method for determining the oxygen uptake of individual embryonated eggs. *J. Immunol.*, **64**: 463-473.
- McLIMANS, W. F., R. A. SIEM, B. C. MARK AND E. PINSKA, 1950. A physiological study of virus parasitism. II. The effect of environmental temperature on the rate of oxygen consumption of normal eggs and eggs infected with Newcastle disease virus. *J. Immunol.*, **64**: 475-485.
- MURRAY, H. A., JR., 1925. Physiological ontogeny. A. Chicken embryos. III. Weight and growth as functions of age. *J. Gen. Physiol.*, **9**: 39-48.
- MURRAY, H. A., JR., 1926. Physiological ontogeny. A. Chicken embryos. XII. The metabolism as a function of age. *J. Gen. Physiol.*, **10**: 337-343.
- PETREN, T., AND A. SOLLBERGER, 1953. Die 24-Stunden Rhythmik des Leberglykogens bei Hühnerembryonen und Küken verschiedenen Alters nebst studien über die Unabhängigkeit der Rhythmik von äusseren Faktoren. *Acta. Med. Scand.*, **145**: suppl. 278, pp. 54-66.
- PITTENDRIGH, C. S., AND V. G. BRUCE, 1957. An oscillator model for biological clocks. In: *Rhythmic and Synthetic Processes in Growth*. Dorothea Rudnick, ed., pp. 75-109. Princeton, Princeton Univ. Press.
- PITTENDRIGH, C. S., AND V. G. BRUCE, 1959. Daily rhythms as coupled oscillator systems and their relation to thermoperiodism and photoperiodism. In: *Photoperiodism and Related Phenomena in Plants and Animals*. R. B. Withrow ed., pp. 475-505. Washington, D. C., American Association for the Advancement of Science.
- SIMPSON, G. G., A. ROWE AND R. C. LEWONTIN, 1960. *Quantitative Zoology*. New York, Harcourt, Brace and Company.
- SOLLBERGER, A., 1965. *Biological Rhythm Research*. New York, American Elsevier Publishing Company, Inc.
- WEBB, H. M., AND F. A. BROWN, JR., 1959. Timing long-cycle physiological rhythms. *Physiol. Rev.*, **39**: 127-161.